Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

12-month safety and efficacy of everolimus with reduced exposure cyclosporine in *de novo* renal transplant recipients

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Keywords
cyclosporine, efficacy, everolimus, renal transplant, safety.

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The results in this manuscript were presented at the American Transplant Congress, Boston, May 2004 (Abstract 504 and 507).

Received: 14 July 2006
Revision requested: 9 August 2006
Accepted: 5 October 2006

doi:10.1111/j.1432-2277.2006.00414.x

Summary

The proliferation signal inhibitor everolimus (Certican), has demonstrated efficacy with full-dose cyclosporine (CsA) (Neoral®). Two multicenter randomized controlled studies were performed to compare 12-month efficacy and safety of everolimus 1.5 and 3.0 mg/day with reduced-dose CsA. Study 1 enrolled 237 *de novo* renal allograft recipients, randomizing 222 nonblack patients to either everolimus 1.5 or 3.0 mg/day, with the Neoral® dose guided by C_2 (monitoring of CsA concentration 2 h after dosing). Study 2 had a similar protocol, with basiliximab included, enrolling 256 recipients and randomizing 243 nonblack patients. In Study 1, there was a lower incidence of acute rejection in nonblack patients on 3 mg/day (16.4%) compared with 1.5 mg/day (25.9%), $P = 0.08$. In Study 2, the inclusion of basiliximab lowered the overall incidence of acute rejection; 14.3% of nonblack patients (3 mg/day) and 13.6% of nonblack patients (1.5 mg/day) had acute rejection by 12 months ($P = 0.891$). Renal function was preserved throughout the study, with no differences observed between groups within studies. Everolimus was well tolerated with no significant differences between doses. Everolimus, in combination with reduced-dose Neoral®, demonstrated efficacy and was well tolerated. Basiliximab allows for utilization of lower doses of everolimus with reduced dosing of Neoral®.

Introduction

The survival of renal allografts from deceased donors is impacted by the events that occur at the time of transplantation and during the first year post-transplant. Previously, factors such as delayed graft function (DGF) and the occurrence of acute rejection were the monumental events post-transplant that impacted allograft survival. Recently, an appreciation has been developed for the association between the level of renal function and the longevity of a renal allograft [1]. Calcineurin inhibitors (CNIs) diminish the rate of acute rejection; however, utilization of these agents entails the potential for concomitant reduction of renal function. Immunosuppressive regimens that provide for the reduction of CNI levels to minimize nephrotoxicity, without increasing the risk of immunological events, may potentially extend the survival of allografts [2].

The novel immunosuppressant everolimus (Certican®; RAD, Novartis Pharma AG, Basel, Switzerland) inhibits the T-lymphocyte proliferative response to cytokine signals [3], thus complementing the inhibitory effect of cyclosporine (CsA) on T-cell-dependent growth factors such as interleukin (IL)-2 [4,5]. *In vitro* and preclinical evidence has demonstrated that everolimus enhances the immunosuppressive action of CsA-based regimens [6–9], and phase III trials in which everolimus was used in combination with full-dose CsA have shown equivalent efficacy to mycophenolate mofetil (MMF) [10,11]. On average, higher serum creatinine levels were seen, therefore studies were designed to decrease CsA levels.

Two prospective, multicenter, randomized studies were designed to evaluate the efficacy and safety of reduced exposure to CsA guided by C_2 monitoring [12] in combination with everolimus (1.5 mg/day or 3 mg/day) and corticosteroids in *de novo* renal transplant recipients. No other inhibitor of cellular proliferation was compared in these trials as equivalent efficacy of everolimus was demonstrated versus MMF in phase III trials [10]. An open-label design was adopted as therapeutic drug monitoring was required to adjust everolimus trough levels to >3 ng/ml. The two studies were undertaken concurrently, with similar protocols other than variations in CsA exposure levels and use of an IL-2 receptor antagonist in one of the trials. Previously the 6-month results of these studies were reported [13]. Study 1 (A2306; $n = 237$) had no induction therapy; in study 2 (A2307; $n = 256$) basiliximab was administered (days 0 and 4). Biopsy-proven acute rejection (BPAR) occurred in 25.0% and 15.2% of patients in the 1.5 and 3 mg/day groups in study 1, and 13.7% and 15.1% in study 2. The incidence of BPAR was significantly higher in patients with everolimus trough <3 ng/ml. There were no significant between-group differences in the composite endpoint of BPAR, graft loss or death, nor any significant between-group differences in adverse events in either study. Median serum creatinine levels in study 1 were 133 and 132 μmol/l at 6 months in the 1.5 and 3 mg/day groups, respectively, and 130 μmol/l in both groups in study 2. Although the trials demonstrated that concentration-controlled everolimus with low-exposure CsA was safe, and provided effective protection against acute rejection with preservation of renal function by 6 months post-transplant, it is important to evaluate whether these conclusions remain valid for the second half of the first year post-transplant: a period of time in which critical events, such as acute rejection, still occur with important frequency.

Materials and methods

Study design

The study design has previously been fully described [13]. The studies were conducted to compare the safety and efficacy of two doses of everolimus and performed in accordance with the Declaration of Helsinki and US Food and Drug Administration guidelines for good clinical practice.

Patients

Adult male or nonpregnant female patients who underwent their primary renal transplantation from a deceased, living-unrelated, or human leukocyte antigen-mismatched living-related donor were enrolled (in study 1, but not in study 2, eligible patients could not have delayed graft function within 24 h).

Immunosuppression

In study 1, all nonblack patients were randomized within 24 h of transplantation to receive 1.5 or 3 mg/day everolimus. Everolimus was administered twice daily simultaneously with CsA, at either 0.75 or 1.5 mg b.i.d. All black patients received 3 mg/day everolimus (1.5 mg b.i.d) based upon pharmacokinetic data that indicate black patients have a higher clearance rate of everolimus than Caucasian patients [14]. Everolimus trough concentrations were measured and the dose was adjusted by 0.5 or 0.75 mg b.i.d. if the trough concentration was <3 ng/ml. Trough concentration was measured 5 days after dose adjustment to ensure the target level was achieved. The dose was reduced if patients could not tolerate full-dose everolimus and discontinued if necessary. In study 2, the same randomization scheme was employed; however, all patients received basiliximab in two doses of 20 mg

intravenously administered within 2 h of transplantation and on day 4 post-transplant.

Cyclosporine (Neoral®; Novartis Pharma AG) was given twice daily in equal divided doses at 12-h intervals, at an initial dose of 8 mg/kg/day in study 1 and of 4 mg/kg/day in study 2. Adjustment of CsA dose to target levels was achieved through monitoring of CsA concentration 2 h after dosing (C_2); a better marker of CsA exposure than trough concentration [12]. Blood CsA (C_2) was measured in whole blood taken 2 h (±10 min) after the morning dose and the CsA dose was adjusted from day 3 to target C_2 ranges that were lowered over time post-transplant. In study 1, target C_2 was 1200 ng/ml (range: 1000–1400 ng/ml) for weeks 0–4; 800 ng/ml (range: 700–900 ng/ml) for weeks 5–8; 600 ng/ml (range: 550–650 ng/ml) for weeks 9–12; and 400 ng/ml (range: 350–450 ng/ml) for months 4–12. In study 2, in which patients also received basiliximab, target C_2 was set lower: 600 ng/ml (range: 500–700 ng/ml) for weeks 0–8 and 400 ng/ml (range: 350–450 ng/ml) from week 9 to month 12. CsA exposure could be reduced in the presence of DGF, if patients received antibodies for steroid-resistant rejection episodes or vascular rejection, or for drug-induced kidney dysfunction.

Intravenous corticosteroids were given according to local transplant center. Oral prednisone was initiated on day 1 at a minimum dose of 20 mg/day and continued for at least 12 months tapered to a minimum of 5 mg/day. In study 2, basiliximab was given according to the standard dose regimen, 20 mg on day 0 (within 2 h before transplantation) and day 4 as an intravenous bolus.

Statistical analysis

The primary endpoint for both studies was renal function, measured by estimated glomerular filtration rate (GFR) (Nankivell formula) [15], calculated creatinine clearance (Cockroft–Gault) [16], and serum creatinine at 12 months. All serum creatinines were measured in a central laboratory to avoid calibration bias between centers. Efficacy endpoints included the first occurrence of either BPAR, graft loss, death, or lost to follow-up. All suspected episodes of acute rejection were recorded. An allograft core biopsy performed within 48 h of suspected rejection was graded according to the 1997 Banff criteria.

Renal function data were analyzed using the intent-to-treat approach based on all data (i.e. including data observed after discontinuation of study medication) as well as an on-treatment analysis. All efficacy analyses were conducted on data from the intent-to-treat population. Comparisons between treatment groups of the proportion of patients experiencing composite efficacy failure and its individual components were made using the Fisher's exact test. Kaplan–Meier estimates of the probability of a first event of efficacy failure within 12 months were performed.

Sample size considerations

The number of patients targeted to be enrolled into the two studies was determined by sample size calculations to simultaneously satisfy two conditions. First, upon fixing a two-sided type I error at 5%, 92 patients per study arm were required, to have 80% power to detect a between-treatment group difference of 25 μmol/l in mean on-treatment creatinine in nonblack patients, assuming a standard deviation (SD) of 60 μmol/l for serum creatinines and a 15% drop out rate by 6 months. Second, upon fixing the type I error rate at 5% and assuming a SD of 60 μmol/l and a true mean value of 150 μmol/l, 78 patients per treatment arm were required for at least 90% power to show that the upper limit of the one-sided 95% confidence interval for mean on-treatment creatinine (in all patients) was <170 μmol/l. A total sample size per study of 216 nonblack patients was chosen.

Results

Baseline characteristics

A total of 237 patients were enrolled in study 1 (112 and 125 in the everolimus 1.5 and 3 mg groups, respectively). Of these subjects, 222 nonblack patients were randomized (112 and 110 in the everolimus 1.5 and 3 mg groups, respectively). All 15 black patients enrolled in study 1 were assigned to the everolimus 3 mg group. A total of 256 patients were enrolled in study 2 (117 and 139 in the everolimus 1.5 and 3 mg groups, respectively), and 243 nonblack patients were randomized (117 and 126 in the everolimus 1.5 and 3 mg groups, respectively). All 13 black patients were assigned to the everolimus 3 mg group. Baseline demographics and background characteristics are shown in Table 1. There were no significant differences between the 1.5 and 3 mg/day treatment groups in either study, other than the inclusion of all black patients in the 3 mg/day everolimus groups.

Immunosuppression

As reported at 6 months, the proportion of patients who had everolimus trough levels less than the target of 3 ng/ml was significantly higher among the patients randomized to the everolimus 1.5 mg/day arms of both studies. By months 6–12, few patients in the everolimus 1.5 and 3 mg groups had everolimus trough levels <3 ng/ml (3–5% and 2%, respectively, in study 1, and 2–3% and

Table 1. Patient demographics and baseline characteristics.

	Study 1 (without basiliximab)		Study 2 (with basiliximab)	
	Everolimus (1.5 mg/day) ($n = 112$)	Everolimus (3 mg/day) ($n = 125$)	Everolimus (1.5 mg/day) ($n = 117$)	Everolimus (3 mg/day) ($n = 139$)
Mean age ± SD (years) (range)	42.5 ± 12.3 (19–67)	42.8 ± 12.8 (19–67)	43.9 ± 12.7 (18–68)	46.3 ± 11. 8 (19–71)
Gender (% male)	70 (62.5%)	67 (53.6%)	81 (69.2%)	87 (62.6%)
Ethnicity				
Caucasian	88 (78.6%)	83 (66.4%)	106 (90.6%)	116 (83.5%)
Black	0	15 (12.0%)	0	13 (9.4%)
Hispanic	13 (11.6%)	14 (11.2%)	4 (3.4%)	4 (2.9%)
Oriental	0	5 (4.0%)	4 (3.4%)	3 (2.2%)
Other	11 (9.8%)	8 (6.4%)	3 (2.6%)	3 (2.2%)
BMI	24.2 ± 4.1	25.0 ± 4.7	25.3 ± 4.3	25.6 ± 5.0
Primary cause of end-stage renal disease				
Glomerular disease	30 (26.8%)	38 (30.4%)	32 (27.4%)	41 (29.5%)
Polycystic disease	16 (14.3%)	15 (12.0%)	14 (12.0%)	23 (16.5%)
Hypertension/nephrosclerosis	12 (10.7%)	21 (16.8%)	4 (3.4%)	12 (8.6%)
Diabetes mellitus	6 (5.4%)	7 (5.6%)	10 (8.5%)	15 (10.8%)
Unknown	24 (21.4%)	19 (15.2%)	19 (16.2%)	10 (7.2%)
Other	13 (11.6%)	15 (12.0%)	24 (20.5%)	22 (15.8%)
Cadaveric donor	67 (59.8%)	82 (65.6%)	79 (67.5%)	107 (77.0%)
Patients with DGF	16 (14.3%)	21 (16.8%)	23 (19.7%)	28 (20.1%)
Mean HLA mismatches				
<3	27 (24.1%)	30 (24.0%)	22 (33.8%)	14 (20.6%)
≥3	84 (75.0%)	93 (74.4%)	43 (66.2%)	53 (77.9%)
Unknown	1 (0.9%)	2 (1.6%)	0	1 (1.5%)
% Patients with panel-reactive antibodies >10%	10.8 ($n = 93$)	5.6 ($n = 106$)	13.7 ($n = 111$)	12.3 ($n = 133$)
Mean cold ischemia time ± SD (hours)				
Cadaveric donor	16.5 ± 5.8	17.6 ± 6.2	16.4 ± 6.5	16.3 ± 6.1
Living donor	1.4 ± 1.4	1.6 ± 2.1	1.3 ± 1.0	1.3 ± 1.3
Mean donor age ± SD (years)	42.4 ± 12.7	40.9 ± 13.9	40.6 ± 13.5	37.9 ± 14.2

BMI, body mass index (in kg/m^2); DGF, delayed graft function; HLA, human leukocyte antigen; SD, standard deviation; NOS, not otherwise specified.

3–4%, respectively, in study 2). Over time, mean daily dosage and CsA levels decreased without significant differences between the arms of both studies (Tables 2 and 3).

Renal function

Renal function as represented by either GFR estimation or by serum creatinine was preserved throughout months 6–12 post-transplant (Table 4). No inter-group comparisons were statistically significant. Proteinuria was detected infrequently in everolimus 1.5 and 3 mg arms (in study 1, 4.5% and 2.4%, respectively, and in study 2, 4.3% and 3.6%; proteinuria was reported as an adverse event at month 12 in both the studies).

Efficacy endpoints

The time to the first efficacy endpoint (BPAR, death, allograft lost, or loss to follow-up) was similar between everolimus 1.5 mg and everolimus 3 mg within both the studies (Fig. 1). The incidence of death or graft loss was low in all patient groups (Table 5), and the incidence of acute rejection was not statistically different between everolimus 1.5 and 3 mg arms within the studies. However in study 2, where all patients were treated with basilimixab, a lower proportion of patients was observed to have an episode of acute rejection. When the analyses were then limited to persons who were randomized, i.e. excluding blacks, there was a trend towards a reduction in the incidence of BPAR in study 1 among nonblacks randomized to 3 mg everolimus compared with 1.5 mg everolimus (16.4% and 25.9%, respectively, $P = 0.08$). In study 2, 13.6% and 14.3% of nonblacks who were randomized to everolimus 1.5 and 3 mg, respectively, had BPAR by 12 months ($P = 0.891$). Most cases of BPAR were mild or moderate in severity; only four cases of BPAR were grade III in study 1 (two in each treatment group) and two cases in study 2 (both in the 3 mg/day group). Note that from month 6 to month 12, the

Table 2. Everolimus dose and trough concentrations by visit window (mean ± standard deviation).

	Everolimus dose and trough concentrations							
	Study 1				Study 2			
	Everolimus (1.5 mg/day) ($n = 112$)		Everolimus (3 mg/day) ($n = 125$)		Everolimus (1.5 mg/day) ($n = 117$)		Everolimus (3 mg/day) ($n = 139$)	
Visit	Everolimus dose (mg/day)	Everolimus C_0 (ng/ml)	Everolimus dose (mg/day)	Everolimus C_0 (ng/ml)	Everolimus dose (mg/day)	Everolimus C_0 (ng/ml)	Everolimus dose (mg/day)	Everolimus C_0 (ng/ml)
Month 6	1.8 ± 0.6 ($n = 86$)	5.2 ± 1.8 ($n = 82$)	2.5 ± 0.7 ($n = 103$)	7.3 ± 3.0 ($n = 98$)	2.4 ± 0.9 ($n = 99$)	6.5 ± 3.8 ($n = 97$)	3.0 ± 0.9 ($n = 118$)	7.6 ± 3.2 ($n = 118$)
Month 9	1.9 ± 0.8 ($n = 81$)	5.8 ± 2.5 ($n = 76$)	2.4 ± 0.7 ($n = 100$)	8.0 ± 4.0 ($n = 92$)	2.4 ± 1.0 ($n = 95$)	7.0 ± 3.2 ($n = 90$)	2.9 ± 1.0 ($n = 117$)	7.5 ± 3.1 ($n = 114$)
Month 12	1.9 ± 1.0 ($n = 79$)	5.7 ± 2.2 ($n = 74$)	2.4 ± 0.7 ($n = 95$)	8.0 ± 4.7 ($n = 89$)	2.4 ± 1.0 ($n = 93$)	7.0 ± 3.0 ($n = 90$)	2.9 ± 1.0 ($n = 116$)	7.6 ± 3.3 ($n = 107$)

Table 3. Cyclosporine (CsA) target C_2 range and dose, trough (C_0), and peak (C_2) concentrations by visit window (mean ± standard deviation).

		Study 1					
		Everolimus (1.5 mg/day) ($n = 112$)			Everolimus (3 mg/day) ($n = 125$)		
Target C_2 (range) (ng/ml)	Visit	CsA dose (mg/kg)	C_2 (ng/ml)	C_0 (ng/ml)	CsA dose (mg/kg)	C_2 (ng/ml)	C_0 (ng/ml)
Month 4-end 400 (350–450)	Month 6	2.1 ± 0.7 ($n = 86$)	533.6 ± 264.8 ($n = 84$)	81.7 ± 59.5 ($n = 80$)	1.9 ± 1.9 ($n = 103$)	597.6 ± 485.2 ($n = 95$)	83 ± 67 ($n = 97$)
	Month 9	2.1 ± 0.8 ($n = 81$)	474.6 ± 213.4 ($n = 78$)	83.8 ± 62.7 ($n = 74$)	1.8 ± 0.6 ($n = 100$)	473.6 ± 229.6 ($n = 90$)	71.3 ± 42.8 ($n = 87$)
	Month 12	2.0 ± 0.8 ($n = 79$)	469.5 ± 347.4 ($n = 77$)	61.1 ± 28.0 ($n = 73$)	1.8 ± 0.7 ($n = 95$)	430.7 ± 155.2 ($n = 92$)	70.5 ± 43.8 ($n = 86$)
		Study 2					
		Everolimus (1.5 mg/day) ($n = 117$)			Everolimus (3 mg/day) ($n = 139$)		
Month 4-end 400 (350–450)	Month 6	2.0 ± 0.8 ($n = 99$)	447.6 ± 159.6 ($n = 96$)	63.7 ± 31.6 ($n = 96$)	1.9 ± 0.7 ($n = 118$)	459.6 ± 207.9 ($n = 117$)	67.7 ± 58.9 ($n = 115$)
	Month 9	1.8 ± 0.65 ($n = 95$)	429.6 ± 160.5 ($n = 92$)	65 ± 31 ($n = 90$)	1.9 ± 0.68 ($n = 117$)	402.4 ± 157.4 ($n = 116$)	62 ± 46 ($n = 111$)
	Month 12	1.8 ± 0.65 ($n = 93$)	430.5 ± 180.9 ($n = 90$)	60 ± 30 ($n = 88$)	1.8 ± 0.65 ($n = 116$)	370.3 ± 166.0 ($n = 109$)	58 ± 54 ($n = 101$)

Table 4. Serum creatinine values, calculated creatinine clearances and estimated glomerular filtration rates for everolimus (patients with at least one assessment in any visit window, including data obtained after discontinuation of study medication).

	Study 1			Study 2		
Months Post-transplant	Median serum creatinine (µmol/l) (mean ± SD)	Median calculated creatinine clearance (ml/min) (mean ± SD)	Median calculated GFR (ml/min) (mean ± SD)	Median serum creatinine (µmol/l) (mean ± SD)	Median calculated creatinine clearance (ml/min) (mean ± SD)	Median calculated GFR (ml/min) (mean ± SD)
Everolimus (1.5 mg/day)						
6	133 (147 ± 104.7) ($n = 105$)	66 (64 ± 24.2) ($n = 105$)	65 (63 ± 19.5) ($n = 102$)	130 (137 ± 49.8) ($n = 113$)	66 (67 ± 23.8) ($n = 113$)	66 (66 ± 18.8) ($n = 107$)
12	131 (140 ± 51.1) ($n = 105$)	65 (65 ± 24.5) ($n = 105$)	64 (64 ± 19.3) ($n = 101$)	129 (140 ± 75.1) ($n = 110$)	64 (67 ± 24.2) ($n = 110$)	68 (65 ± 17.4) ($n = 104$)
Everolimus (3 mg/day)						
6	132 (140 ± 53.1) ($n = 112$)	62 (62 ± 21.6) ($n = 112$)	62 (62 ± 18.3) ($n = 111$)	130 (136 ± 42.3) ($n = 127$)	63 (64 ± 20.3) ($n = 127$)	67 (65 ± 16.0) ($n = 123$)
12	130 (137 ± 48.7) ($n = 111$)	63 (64 ± 22.6) ($n = 111$)	63 (62 ± 17.7) ($n = 108$)	128 (136 ± 44.5) ($n = 124$)	64 (64 ± 19.6) ($n = 124$)	68 (65 ± 16.2) ($n = 122$)

GFR, glomerular filtration rate; SD, standard deviation.

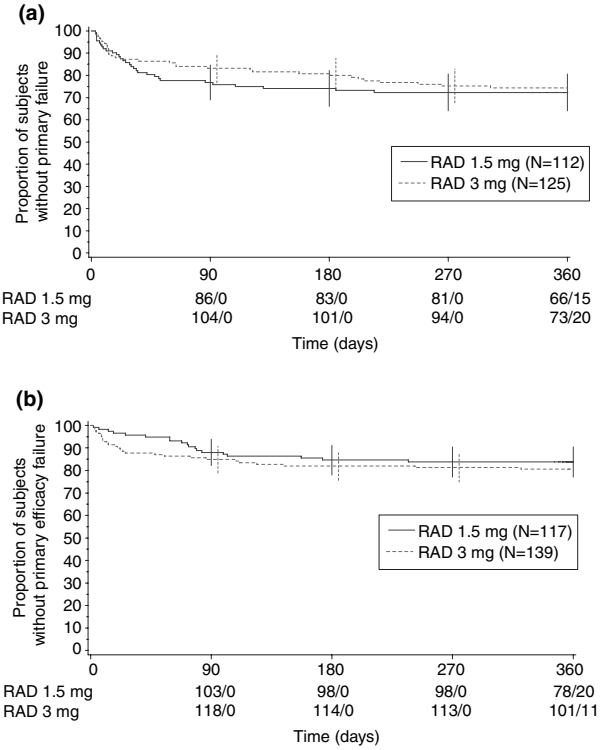

Figure 1 (a) Time to the first efficacy endpoint [biopsy-proven acute rejection (BPAR), death, allograft loss, or lost to follow-up] in study 1. (b) Time to the first efficacy endpoint (BPAR, death, allograft loss, or lost to follow-up) in study 2.

number of black patients in study 1 ($n = 15$) experiencing primary efficacy failure increased from four (26.7%) to seven (46.7%), and black patients in study 2 ($n = 13$) experiencing primary efficacy failure increased from three (23.1%) to five (38.5%). The number of black patients experiencing BPAR were four (26.7%) in study 1 and three (23.1%) in study 2 at 6 months, and six (40.0%) in study 1 and four (30.8%) in study 2 at 12 months. The limited number of black patients enrolled into these trials does not provide for reliable conclusions to be made about unique effects of everolimus in different ethnic groups.

Safety

The most frequent adverse event was the occurrence of urinary tract infections in study 1 (30.4% and 31.2% in the 1.5 mg/day and 3 mg/day groups, respectively) and hyperlipidemia NOS in study 2 (37.6% and 33.1%in the 1.5 mg/day and 3 mg/day groups, respectively). Common adverse events are shown in Table 6. The incidence of adverse events was similar to those reported at 6 months of follow-up. The point prevalence of diabetes mellitus by

Table 5. Efficacy-related events at 12 months (intent-to-treat analyses).

	Study 1 (without basiliximab)			Study 2 (with basiliximab)		
	Everolimus (1.5 mg/day) (n = 112)	Everolimus (3 mg/day) (n = 125)	P*	Everolimus (1.5 mg/day) (n = 117)	Everolimus (3 mg/day) (n = 139)	P*
Efficacy failure†	31 (27.7%)	32 (25.6%)	0.769	19 (16.2%)	27 (19.4%)	0.624
Biopsy-proven acute rejection	29 (25.9%)	24 (19.2%)	0.274	16 (13.7%)	22 (15.8%)	0.725
Allograft loss/death	6 (5.4%)	10 (8.0%)	0.450	2 (1.7%)	7 (5.0%)	0.187
Allograft loss	6 (5.4%)	4 (3.2%)	0.523	2 (1.7%)	7 (5.0%)	0.187
Death	1 (0.9%)	6 (4.8%)	0.123	0	2 (1.4%)	0.502
Lost to follow-up	0	1 (0.8%)	1.000	1 (0.9%)	2 (1.4%)	1.000

*Fisher's exact test.
†BPAR, graft loss, death, or lost to follow-up.

Table 6. Number (%) of patients reporting common adverse events (AEs) by 12 months (safety analyses).

	Study 1 (without basiliximab)		Study 2 (with basiliximab)	
	Everolimus (1.5 mg/day) (n = 112)	Everolimus (3 mg/day) (n = 125)	Everolimus (1.5 mg/day) (n = 117)	Everolimus (3 mg/day) (n = 139)
Any infection	72 (64.3%)	81 (64.8%)	73 (62.4%)	90 (64.7%)
Infection reported as serious AE	18 (16.1%)	18 (14.4%)	20 (17.1%)	19 (13.7%)
Malignancy	2 (1.8%)	2 (1.6%)	3 (2.6%)	3 (2.2%)
Lymphocele	17 (15.2%)	10 (8.0%)	12 (10.3%)	10 (7.2%)
Total cholesterol ≥9.1 mmol/l (351 mg/dl)	25 (22.3%)	33 (26.4%)	24 (20.7%)	31 (22.3%)
Triglycerides ≥8.5 mmol/l (752 mg/dl)	4 (3.8%)	11 (8.8%)	7 (6.0%)	12 (8.6%)
Blood glucose >13.9 mmol/l (250 mg/dl)	12 (10.7%)	13 (10.4%)	10 (8.6%)	12 (8.6%)

3 months post-transplant, defined as the measurement of at least one fasting glucose ≥126 mg/dl [17], was infrequent – eight patients in the everolimus 1.5 mg group and 10 patients in the everolimus 3 mg group in study 1, and 15 patients in the everolimus 1.5 mg group and 19 patients in the everolimus 3 mg group.

Discussion

Everolimus is a novel immunosuppressive agent proliferation signal inhibitor that demonstrated efficacy in phase III trials compared with MMF [10]. Observations from the randomized trials reported here confirmed the continued efficacy during the first year post-transplant of the two doses of everolimus, with or without basilimixab, in combination with Neoral®, having the dosage optimized by C_2 monitoring.

Reduction of the dosage of CNIs is one strategy to minimize the nephrotoxicity of these agents [2]. The use of the antiproliferative agent everolimus allowed for the reduction of CsA dosage, while levels of CsA were optimized by the measurement of C_2 levels; the preferred strategy in comparison with monitoring CsA serum trough levels. Adjusting CsA dosages by C_2 levels was associated with average trough CsA levels at 6, 9 and 12 months; much lower than traditional target levels during these months (by approximately 57%). As a result of lower exposure to CsA, mean and median serum creatinine values at month 12 were low, and stable from months 6 to 12. The patients within these studies had reduced exposures of CsA with doses adjusted according to predefined C_2 levels, hence, the CsA exposures were significantly lower in comparison with other phase III trials that used full-dose Neoral® [18]. In addition these patients had lower serum creatinines with corresponding higher calculated creatinine clearances, and estimated GFRs, in comparison with the phase III trials that used full-dose Neoral® adjusted by trough levels [10,19]. In addition, this improved renal function (relative to the phase III studies) was achieved despite CsA C_2 levels being higher than the target ranges.

Mean everolimus trough levels increased post-transplantation, and were stabilized through month 6, and increased at months 9 and 12. Although at months 6–12, few patients in the everolimus 1.5 and 3 mg groups had everolimus trough levels <3 ng/ml (3–5% and 2%,

respectively), the incidence of everolimus trough levels <3 ng/ml was significantly higher in the everolimus 1.5 mg group early post-transplantation. In both groups, CsA C_2 and trough levels decreased over time with no significant between-group differences in the incidence of patients within the C_2 target ranges observed. The efficacy of the two doses of everolimus, with or without the administration of basiliximab, on the incidence of allograft loss and BPAR persisted throughout the entire first year and was similar to the phase III trials. There was a trend among nonblack recipients in the reduction of the incidence of acute rejection associated with use of 3 mg of everolimus compared with 1.5 mg in the absence of induction therapy. The lower overall incidence of acute rejection in the second study is likely attributable to the inclusion of basiliximab in the immunosuppressive regimen [20,21]. The reduction in rates of acute rejection associated with basiliximab is even more significant when placed into the context that these rate reductions also occurred in patients who were randomized to the lower dosage of everolimus with concomitant low levels of Neoral®. The rates of adverse events in such an immunosuppressive protocol might be anticipated to be lower; however, there were no statistically significant differences between the two studies within this period of time post-transplant. Largely, there was no significant change in the number of adverse events observed in comparison with the first 6 months of therapy.

Overall, the combination of everolimus dosing with therapeutic drug monitoring and reduced-dose Neoral®, guided by C_2 monitoring, and corticosteroids continued to be favorable with regards to safety, tolerability, and efficacy throughout the first year post-transplant. The preservation of renal function associated with the immunosuppressive regimens in these studies and the impact on long-term allograft survival will need to be confirmed with longer follow-up. Reduction of complications related to the long-term intensity of immunosuppression by the use of a lower dosage of everolimus and C_2-guided Neoral® in combination with basiliximab will also require further investigation.

Acknowledgements

We would like to acknowledge the collaboration and commitment of all A2306 (study 1) and A2307 (study 2) investigators and their staff, without whom the present paper would not have been possible. The studies were funded by a grant from Novartis Pharma AG.

Appendix

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